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Effect of Neonicotinoid Acetamiprid and Imidacloprid Insecticides on Antioxidant Peroxidase Activity in Earthworm *Eisenia fetida*

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ABSTRACT: Peroxidase, an antioxidant enzyme, is important in eliminating excess reactive oxygen species from earthworm cells. Insecticides such as neonicotinoid acetamiprid and imidacloprid are becoming more popular by the day in order to increase crop yields. The major goal of this study is to see how different doses of imidacloprid and acetamiprid affect the antioxidant enzyme peroxidase activity in earthworms *Eisenia fetida* and maintaining the right environment for properly measuring the enzymatic activities estimation was extremely challenging because the enzymatic activity of earthworms varied rapidly. During the current experiment, three dosages of acetamiprid (0.145 µg, 0.165 µg, and 0.188 µg) and imidacloprid (0.134 µl, 0.195 µl, and 0.280 µl) were tested on direct exposure in vials with a diameter of 3mm and 8 cm length. After 48 hours of exposure to acetamiprid, peroxidase activity was 0.775 and 0.858Umg^{-1} protein at concentrations of 0.165 µg and 0.188 µg respectively, and 0.805 and 0.885Umg^{-1} protein at 0.195 and 0.285 µl concentrations of imidacloprid respectively. After 24 and 48 hours, peroxidase enzyme activities were 0.633 and 0.638Umg^{-1} proteins, respectively, in the control group. The peroxidase activity of an earthworm is directly related to the concentration and exposure time of these two neonicotinoid insecticides; as the doses of both pesticides increased, the peroxidase activities increased as well, indicating the need to limit pesticide use to protect soil invertebrate flora. It is critical to investigate the impact of neonicotinoid insecticides on earthworm antioxidant activities in order to reduce insecticide overuse and ensure the future conservation of soil invertebrate flora. As a result, similar studies should be conducted in situ and ex-situ experiment in various areas on a regular basis to ensure biodiversity conservation and sustainable use.

Keywords: Acetamiprid, imidacloprid, neonicotinoid, insecticides, *Eisenia fetida*, POD

INTRODUCTION

Neonicotinoids are the most common type of pesticide, and they're used all around the world as selective agonists for insect nicotinic acetylcholine receptors. Apart from their application in agriculture in the form of granules or foliar sprays, they have also been used to control household insects such as termites and cockroaches. They're also utilised to control ectoparasites in veterinary medicine. Because of their structural similarities to nicotine, these compounds are indicated as organophosphate alternatives because of their particular mode of action (Saha *et al.*, 2017; Wang *et al.*, 2015b), which suppresses nerve impulse transmissions in insects (Wang *et al.*, 2015a; Yamamoto, 2012). Neonicotinoids are safer for other organisms because of their strong resemblance in insects that have nicotinic acetylcholine receptors. However, because of their broad range of action, some neonicotinoids may have an impact on organisms that aren't intended to be affected (Miles *et al.*, 2017; Han *et al.*, 2019; Rico *et al.*, 2019). Because of its decreased toxicity, acetamiprid, a neonicotinoid, has been recommended as a global organophosphate replacement (Enrico *et al.*, 2019). Acetamiprid, one neonicotinoid in particular, is a systemic chloronic chemical with significant efficacy against insects including white flies and aphids (Saha *et al.*, 2017, Renaud *et al.*, 2018). The number of earthworms in a given area of soil indicates the health of the ecosystem and the level of environmental safety. Earthworms play an important role in increasing crop output in agricultural settings, where synthetic pesticides such as acaricides, fungicides, herbicides, and insecticides are employed in large quantities to manage hazardous pests. Earthworms

have been shown to be useful soil pollution bioindicators because they are sensitive, easy to grow and maintain, and can be used to research a variety of toxins (Genazio Pereira *et al.,* 2017). *Eisenia fetida* has adapted to thrive in rotting vegetables, leaf litter, and dung, making it perfect for vermicomposting. Vermicompost is an organic fertiliser created by worms like *Eisenia fetida* that contains humus and a good amount of nutrients that plants can ingest without hurting their vegetative growth.

Antioxidant enzymes and immune cells were researched as endpoints because they show sensitivity to doses below acute toxicity thresholds, which is crucial for early detection of insecticide use and the protection of both biota and ecosystems (Gomes *et al.,* 2019; Pereira *et al.,* 2019; Zhang *et al.,* 2013). Enzymes and proteins linked to oxidative stress, such as catalase, glutathione S-transferase, and glutathione, have been used to assess environmental neocotinoid contamination because of their quick response, ease of testing, and high sensitivity at low contaminant concentrations (Li *et al.,* 2018; Wang *et al.,* 2015a; Zhang *et al.,* 2014).

Zhang *et al.,* 2014 found that imdacloprid (0.2, 0.66, 2, and 4 mg/kg) had an effect on the antioxidant defence system of *Eisenia fetida* on the 1st, 7th, and 14th days, with catalase (CAT) activity significantly increasing at concentrations of 0.2, 0.66, and 2 mgkg-1, but a slight decline in CAT activity at 4 mgkg-1, whereas POD activities increased at doses of 0.2, 0. To control soil-borne pests, pesticides are either applied directly on the soil or runoff from foliar sprays is deposited on the soil, and these pesticides affect epigeic earthworms *Eisenia fetida* directly or indirectly (Gupta *et al.,* 2011). Boruah *et al.,* 2019 discovered that using *Eisenia fetida* to bio convert citronella bagasse results in a better final product as vermicompost.

MATERIALS AND METHODS

The experiment took place at Chaudhary Charan Singh Haryana Agricultural University in Hisar from July to September 2020.

A. Preparation of earthworm tissue homogenates by method of Jeyanthi et al., (2016)

The earthworms were treated with different concentrations of imidacloprid (0.134 µl/cm^2, 0.195µl/cm^2, and 0.285 µl/cm^2) and acetamiprid (0.145 µg/cm^2, 0.165 µg/cm2, and 0.188 µg/cm^2) for the antioxidant defence system. They were removed from the vials and gut cleaned earthworm tissue was placed into a prechilled mortar and pestles under ice-cold conditions in 5 For further investigation, the supernatant was kept at 60°C.

B. The protein content was estimated in each earthworm sample using the method of Lowry et al., (1951)

In 20 ml of NaOH, 1 gm/ml of material was homogenised (0.5M). The homogenate was placed in a centrifuge tube and spun for 10 minutes at 3500 rpm. In a separate tube, the supernatant was collected. To make the final volume of 5ml, 4 mL distilled water + 1.0 mL supernatant, in the same way; a 0.2mg standard protein solution (bovine serum albumin) was made. Each tube received 5ml of alkaline solution, which was thoroughly mixed before being left at room temperature for 10 minutes. After that, each tube received 0.05ml of weak Folin Ciacalteau reagent, which was combined immediately to produce a blue colour. Using a spectrophotometer, against a blank reagent, the absorbance was measured at 750 nm (UV-VIS-NIR Spectrophotometer, Varian Cary-5000). The standard BSA curve was used to extrapolate the protein concentration in each sample.

C. Estimation of peroxidase activity (POD)

Song *et al.,* (2009) assessed the rate of guaiacol oxidation in the presence of H_2O_2 at 470 nm to calculate POD. Fill a cuvette with 2.15 ml potassium phosphate buffer (0.1M, pH 7.0), 0.6 ml guaiacol (1 percent), and 0.1 ml enzyme extract using a pipette. Then 25 µl of H_2O_2 (100 mM) were added. The solution had been thoroughly mixed, and the transmission at 470 nm had been set to 100%. For 3 minutes, the rise in absorbance was monitored every 15 seconds. The change in optical density (O.D.) was used to estimate enzyme activity using a molar extinction value of 26.6 mM^{-1} cm^{-1} for guaiacol oxidation. One activity unit of POD was defined as the amount of enzyme that caused a 0.01 absorbance unit per minute rise, and the results were expressed as Umg^{-1} protein.

For the lab investigation, the experimental design was a completely randomized block with four replicates. CRD (in vitro) computed a critical difference (CD) between the treatments using the software "OPSTAT" created at CCS Haryana Agriculture University, Hisar.

RESULTS AND DISCUSSION

Experiments were conducted with three dosages of acetamiprid and imidacloprid. The earthworm's POD activity was affected by pesticide exposure and concentrations. After 24 and 48 hours, POD activities were calculated to be 0.633 and 0.638Umg^{-1} proteins in the control group. POD activities of 0.775 and 0.858 Umg^{-1} proteins at doses of 0.165 µg and 0.188 µg were reported after 48 hours of exposure with acetamiprid treatment, whereas POD activity of 0.763 Umg^{-1} proteins at a concentration of 0.188 µg was seen after 24 hours of exposure with acetamiprid treatment (Table 1).

Table 1: Effect of acetamiprid exposure on POD activity in adult *Eisenia fetida.*

Exposure time	POD activity U/mg protein@ three doses of acetamiprid				
	Control	0.145 µg	0.165µg	0.188 µg	Mean
24hr	0.633	0.648	0.710	0.763	0.688
48hr	0.638	0.740	0.775	0.858	0.753
Mean	0.635	0.694	0.742	0.810	
C.D (at 0.05%)=0.065,SE(d)=0.031,SE(m) = 0.022, F= 11.54, D.F=3,Significance value=0.00007, at treatment					
C.D. (at 0.05%) = 0.046, SE(d) =0.022, SE (m) =0.016, F=8.16 , D.F=1 Significance value = 0.00868, at time					

POD activities were 0.603 and 0.628 Umg^{-1} protein for imidacloprid-treated earthworms and 0.603 and 0.628 Umg^{-1} protein for control earthworms at 24h and 48h, respectively. POD activities in earthworms after 48 hours of imidacloprid exposure were 0.805 and 0.885Umg^{-1} protein at 0.195 and 0.280 µl doses, respectively. At a concentration of 0.285 µl imidacloprid, the result after 24 hours was 0.788 Umg^{-1} (Table 2). The statistical analysis demonstrated that acetamiprid and imidacloprid had a significant impact on POD activity.

Table 2: Effect of Imidacloprid exposure on POD activity in adult *Eisenia fetida.*

Exposure time	POD activity U/mg protein @ three doses of imidacloprid				
	Control	0.134 µl	0.195 µl	0.285 µl	Mean
24hr	0.603	0.658	0.710	0.788	0.689
48hr	0.628	0.718	0.805	0.885	0.759
Mean	0.615	0.688	0.757	0.836	
C.D (at 0.05%)=0.083,SE(d)=0.040,SE(m)=0.028, F=11.18, D.F = 3, Significance value =0.00009, at treatment					
C.D. (at 0.05%) = 0.059, SE(d) =0.028, SE(m) =0.020, F=7.17, D.F = 1 Significance value =0.01311, at time					

The activities of three major antioxidant defence enzymes, Super Oxide Dismutase, Peroxidase, and Catalase, were studied at varied pesticide dosages by Liu *et al.,* (2017). Hydrogen peroxide, superoxide radical, and hydroxyl radicals are examples of reactive oxygen species (ROS), which destroy cellular components and disrupt an organism's physiological and metabolic activities. These three enzymes are part of an antioxidant system that helps protect against ROS produced by pesticide stress (Zelikoff *et al.,* 1996). Variations in this enzymatic activity serve as biomarkers and early warning indices for the presence of pollutants in the environment (Fatima and Ahmad, 2005; Aina *et al.,* 2007). The activity of an antioxidant enzyme in earthworms is altered due to stress caused by neonicotinoid insecticides, according to a study by Parveen *et al.,* 2021. So, at various dosages of imidacloprid, the activity of a key antioxidant defense enzyme called Superoxide Dismutase (SOD) was measured in *Eisenia fetida*. The results of SOD activity showed that it was entirely dependent on time and pesticide concentrations. During the experiment, three dosages of imidacloprid were utilized to determine enzymatic activity.

POD activities in the control were 0.633 and 0.638Umg^{-1} protein after 24 and 48 hours, respectively, whereas in the acetamiprid treatment, POD activities of 0.775 and 0.858 Umg^{-1} protein at doses of 0.165 µg and 0.188 µg were observed after 48 hours, whereas POD activity of 0.763Umg^{-1} protein at a dose of 0.188 µg was observed after 24 hours. After 48 hours of treatment with imidacloprid, POD activity in earthworms were 0.805 and 0.885Umg^{-1} protein at dosages of 0.195 and 0.285 µl, respectively, and after 24 hours of treatment with imidacloprid, it was 0.788 Umg^{-1} at a dose of 0.285 µl.

Zhang (2014) observed that POD activities rose at dosages of 0.20, 0.66, and 2$mgkg^{-1}$ of imdacloprid, which followed a similar pattern of results. POD has the ability to scavenge hydrogen peroxide by oxidizing co substrates such as ascorbate and guaiacol, and has been shown to protect *E. fetida* from oxidative stress induced by imidacloprid at low concentrations for short periods of time. POD and CAT activities rose in lockstep with increasing clothianidin neonicotinoide pesticide dosages, implying that POD and CAT are involved in the removal of excess ROS (Tong *et al.,* 2017).

POD and CAT enzymes are widely distributed in peroxisomes, which degrade hydrogen peroxide into water and oxygen, according to Wu *et al.,* (2012). Few studies (Kammenga *et al.,* 2000; Rodrguez and Hernández 2007) looked at the oxidative stress sensitivities of POD and CAT enzymes and how they responded.

CONCLUSION

Pesticides have a greater impact on *Eisenia fetida*, and the study found that POD activities are completely reliant on pesticide concentrations and direct exposure time. The activities of POD enzyme after 24hr and 48hr exposure rose as acetamiprid and imidacloprid concentrations increased, indicating the need to limit pesticide use to protect soil invertebrate flora.

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